probes while the claims of this application are drawn to probes having a double-stranded region...continuous stacking hybridization taught by Khrapko would not be possible without a partially single-stranded probe" (see Office Action mailed June 19, 2001, paragraph 5). It is further alleged that single-stranded probes become partially double-stranded during hybridization and that the instant claims are generic with respect to the time before hybridization in which the partially single-stranded probe is formed. The Examiner also alleges that "any single base" can be considered a random sequence. This rejection is respectfully traversed.

The Claims

Claims 1-5, 65-69 and 111-113 are directed to methods of creating an array of probes containing a double-stranded region and a single-stranded region having a random sequence which include steps of synthesizing a first set of nucleic acids containing a constant sequence and a random sequence and hybridizing the first set of nucleic acids with a second set of nucleic acids each containing a sequence complementary to the constant sequence of the first nucleic acid. Claims 1, 2, 5 and 65-69 specify that the double-stranded region comprises the constant sequence. Claims 111 and 112 specify that the random sequence is of length R. Claims 113, 3 and 4 specify that the constant sequence is of length C at a 3′ terminus and that the random sequence is of length R at a 5′ terminus.

Claims 70-79, 114 and 115 are directed to an array of nucleic acid probes that have a double-stranded portion and a single-stranded portion containing a random nucleotide sequence. Claims 70-79 and 115 specify that the random nucleotide sequence is of length R. Claim 114 specifies that the double-stranded portion comprises a constant sequence.

Claims 89-94 and 116 are directed to a solid support comprising an array of nucleic acid probes that have a double-stranded portion and a single-stranded portion containing a random nucleotide sequence. Claims 89-94 specify that

the random nucleotide sequence of the probes is of length R. Claim 116 specifies that the double-stranded portion of the probes comprises a constant sequence.

Claims 95-110 and 117-120 are directed to methods of sequencing a target nucleic acid including steps of hybridizing target nucleic acid that is at least partly single-stranded to an array of nucleic acid probes that have a double-stranded portion and a single-stranded portion containing a random nucleotide sequence and either detecting the hybridized target nucleic acid for sequencing thereof or determining a hybridization pattern whereby the target nucleic acid is sequenced by analyzing the hybridization pattern. Claims 117 and 119 specify that the random nucleotide sequence is of length R. Claims 118 and 120 specify that the double-stranded portion comprises a constant sequence.

Claims 80-88 and 121-122 are directed to methods of detecting a target nucleic acid in a sample including steps of contacting the sample with an array of probes that have a double-stranded portion and a single-stranded portion containing a random nucleotide sequence and identifying hybrids to detect the target nucleic acid. Claim 121 specifies that the random nucleotide sequence is of length R. Claim 122 specifies that the double-stranded portion comprises a constant sequence.

Cited Art

Khrapko *et al.*

Khrapko describes a technique of DNA sequencing by hybridization with an oligonucleotide matrix (SHOM) and experiments to test the method on a short DNA fragment (17 nucleotides) prior to a full-scale trial of the method. No full scale trial is described in the reference. The efficiency of SHOM is stated to depend on the ability to sort out effectively perfect duplexes from imperfect duplexes (i.e., containing base pair mismatches) which can be achieved by comparing each temperature-dependent dissociation curve of the duplexes

formed by DNA and the immobilized oligonucleotides, with standard dissociation curves for perfect oligonucleotide duplexes.

The method is stated to require a determination of the dissociation curve for all hybridizing oligonucleotides in order to differentiate perfect duplexes from imperfect duplexes (i.e., containing base pair mismatches). A comparison of temperature-dependent dissociation curves of the duplexes formed by DNA and each of the immobilized oligonucleotides, with standard dissociation curves for perfect oligonucleotide duplexes, is used to identify perfect duplexes and/or the degree of mismatch in the hybrids, and thereby the sequence of the target.

Khrapko states that the efficiency of SHOM can be increased by using continuous stacking hybridization (CSH) to resolve ambiguities at branch points. CSH is described as being based on the **simultaneous** hybridization of **two oligonucleotides to a larger oligonucleotide** (emphasis added, see page 385, first full paragraph in left column). Figure 8 of Khrapko is said to illustrate this effect.

Figure 8 of Khrapko shows dissociation curves for four different hybridization products. In each reaction to generate a hybridization product, **three separate oligonucleotides were simultaneously hybridized**. Specifically, a ^{32}P-labeled 5-mer and the "test" 17-mer were simultaneously hybridized with an immobilized oligonucleotide (3 different 8-mers and one 7-mer were immobilized in the matrix). The hybridization products were subjected to washes of increasing temperature in order to generate the dissociation curves shown. It is concluded that (1) the 5-mer makes a stable duplex when hybridized to a complementary 17-mer together with a particular immobilized 8-mer [d(GTCGTTTT)] due to the continuous stacking contact; and (2) the stability of the 5-mer duplex decreases if stacking is disrupted by nucleotide displacement, a gap, or terminal nucleotide mismatch.

Drmanac *et al.*

Drmanac describes experiments designed to investigate possible DNA hybridization conditions that may permit discrimination between perfectly matched duplexes and duplexes with a single mismatch. In these experiments, single-stranded DNA was spotted on a membrane and then hybridized with an oligomer probe end-labeled with ^{32}P. Autoradiographs of the membranes were made to generate a "fingerprint" of an array of clones, although Drmanac teaches liquid scintillation methods can be employed to quantitate binding.

Experiments with model oligonucleotides and an M13 vector and its derivatives were used as a system to demonstrate hybridization to short oligonucleotide probes of 6, 7 or 8 nucleotides. To allegedly show the general utility of the proposed conditions, Drmanac examined hybridization of 4 heptamers, 10 octamers and 14 additional probes up to 12 nucleotides long in the M13 system. To allegedly show the utility of the method in fingerprinting unknown clones for the presence of a short sequence, three probes, each eight nucleotides long, were tested on a collection of 51 plasmid DNA dots made from a library in Bluescript vector. It is concluded in Drmanac that using low-temperature conditions, sufficient difference in hybridization signal was obtained between a dot containing the perfect and mismatched targets and a dot containing only the mismatched targets.

Analysis

All of the pending claims have been rejected as a "group" in the Office Action because CSH taught by Khrapko allegedly would not be possible without a partially single-stranded probed which is formed at the same time as the hybridization is carried out. As set forth above and discussed in detail below, the pending claims are directed to (1) arrays of specific probes of a specific structure [claims 70-79, 114-115]; (2) solid supports comprising the arrays [claims 89-94, 116]; (3) specific methods of creating an array of probes which

set out specific steps to produce probes of a specific structure (i.e., having a double-stranded region and a single-stranded region containing a random sequence [claims 1-5, 65-69, and 111-113]; (4) methods of sequencing a nucleic acid [claims 95-110, 117-120]; and (5) methods of detecting a target nucleic acid [claims 80-88, 121-122].

The rejection of the claims on this basis is traversed for the reasons set forth below. In addition, when the claims, which are directed to different methods and compositions, are separately considered, it is clear that many of the claims are not in the purview of the single basis for rejection of all the claims. Accordingly, the traversal of the rejection is discussed below with reference to each of the particular methods and compositions claimed in the instant claims.

1. The arrays of probes of specific structure as set out in claims 70-79 and 114-115 are not taught or suggested by the cited references.

Claims

Claims 70-79, 114 and 115 are directed to an array of nucleic acid probes that have a double-stranded portion and a single-stranded portion containing a random nucleotide sequence. Claims 70-79 and 115 specify that the random nucleotide sequence is of length R. Claim 114 specifies that the double-stranded portion comprises a constant sequence.

Differences between the claimed array of probes and the cited references

Khrapko describes a technique (SHOM) for sequencing nucleic acids by hybridization of a target single-stranded nucleic acid sequence to single-stranded probes. In experiments described in the reference, single-stranded 8-mers were immobilized to a polyacrylamide-covered glass plate. Four single-stranded 17-mers differing by a single base substitution were separately hybridized to the immobilized 8-mers, each of which was complementary to a portion of one of the 17-mers. The hybridizations would thus form perfect as well as imperfect (single mismatches) duplexes. It is suggested in Khrapko that continuous stacking hybridization (CSH) could increase the fidelity of SHOM. The reference

describes CSH as the simultaneous hybridization of single-stranded target DNA with immobilized single-stranded octanucleotides **in the presence of** labeled selected single-stranded pentanucleotides to form a continuously stacked perfect duplex of 13 base pairs. Drmanac utilizes labeled single-stranded probes hybridized to target nucleic acid (see, e.g., p. 528, left column, third full paragraph). Neither Khrapko nor Drmanac teach or suggest an array of probes containing a double-stranded portion and a single-stranded portion including a random sequence as claimed in the instant application. Such a composition containing nucleic acid molecules of the specific structure set forth in the claimed arrays simply is not taught, suggested or the result of the methods described in the cited references.

Analysis

The Office Action has failed to set forth a case of *prima facie* obviousness

Relevant law

In order to set forth a *prima facie* case of obviousness under 35 U.S.C. §103: (1) there must be some teaching, suggestion or incentive supporting the combination of cited references to produce the claimed invention (*ACS Hospital Systems, Inc. v. Montefiore Hospital*, 732 F.2d 1572, 1577, 221 U.S.P.Q. 329, 933 (Fed. Cir. 1984)) and (2) the combination of the cited references must actually teach or suggest the claimed invention. Further, that which is within the capabilities of one skilled in the art is not synonymous with that which is obvious. *Ex parte Gerlach*, 212 U.S.P.Q. 471 (Bd. App. 1980). Obviousness is tested by "what the combined teachings of the references would have suggested to those of ordinary skill in the art" (*In re Keller*, 642 F.2d 413, 425, 208 U.S.P.Q. 871, 881 (CCPA 1981)), but it cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination (*ACS Hosp. Systems, Inc. v Montefiore Hosp.*, 732 F.2d 1572, 1577, 221 U.S.P.Q. 329, 933 (Fed. Cir. 1984)). "To imbue one of ordinary skill in the art with knowledge

of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher" *W.L. Gore & Associates, Inc. v. Garlock Inc.*, 721 F.2d 1540, 1553, 220 U.S.P.Q. 303, 312-13 (Fed. Cir. 1983). Importantly, **all claim limitations** must be taught or suggested by the prior art to establish that claims are *prima facie* obvious. *See, e.g.,* MPEP 2143.03 and *In re Lowry*, 32 F.3d 1579, 32 U.S.P.Q.2d 1031 (Fed. Cir. 1994), citing *In re Gulack*, 703 F.2d 1381, 217 U.S.P.Q. 401 (Fed. Cir. 1983), citing *In re Royka*, 490 F.2d 981, 180 U.S.P.Q.2d 580 (CCPA 1974).

The combination of teachings of Khrapko *et al.* with the teachings of Drmanac *et al.* does not result in the instantly claimed array of probes.

Neither Khrapko nor Drmanac, individually or in combination, teach or suggest an array of probes that have a double-stranded portion and a single-stranded portion containing a random nucleotide sequence. Although it is alleged in the Office Action that "continuous stacking hybridization taught by Khrapko would not be possible without a partially single-stranded probe," such an assertion has no basis in the very explicit description of CSH provided in Khrapko (see page 385 and Figure 8 of Khrapko). The description clearly states that in CSH, "two oligonucleotides are simultaneously hybridized to a longer one" and that the "5-mer makes a stable duplex when hybridized to a complementary 17-mer **together with** immobilized 8-mer." Thus, **three** oligonucleotides are simultaneously hybridized to give a "continuously stacked duplex of 13 base pairs" (see page 376 of Khrapko). The resulting duplex is **not** of the structure specified for probes in the claimed arrays. The resulting duplex does not contain a double-stranded portion and a single-stranded portion including a random sequence. It is a **duplex**. The claimed array of probes is not suggested nor is a method of generating such an array of probes taught or suggested in Khrapko. Thus, there is no support or basis in Khrapko for the assertion in the Office Action that "continuous stacking hybridization taught by

Khrapko would not be possible without a partially single-stranded probe." Furthermore, such an assertion is irrelevant to a consideration of the patentability of the compositions claimed in claims 70-79, 114 and 115 as an array of probes of the specific structure specified in the claimed compositions is not taught or suggested in Khrapko whether CSH is possible or not without a partially single-stranded probe. The structure generated in the CSH technique described by Khrapko is a continuously stacked 13-base pair duplex formed from simultaneous hybridization of three oligonucleotides. It does not contain a double-stranded portion and a single-stranded portion including a random sequence as specified in the claimed arrays. Drmanac does not cure this deficiency. Although Drmanac describes hybridization of labeled single-stranded probes to a short oligonucleotide acid (see, e.g., p. 528, left column, third full paragraph), the resulting structures are not an array of probes that contain a double-stranded portion and a single-stranded portion that includes a random sequence of length R or a double-stranded portion containing a constant sequence as specified in the claimed arrays.

In conclusion, the cited references, alone or in combination, fail to teach or suggest an array of probes having the specific structure set forth in claim 70 or 114. In addition, the cited references, alone or in combination, fail to teach or suggest such an array of probes wherein the probes are fixed to a solid support (claims 74-76), labeled (claims 77 and 78) or are DNA, RNA or PNA, or a combination thereof (claim 79). Furthermore, Khrapko and Drmanac, alone or in combination, fail to teach or suggest such an array of probes wherein the random nucleotide sequence is of length R (claim 70), or wherein the array comprises about 4^R different probes (claim 71) or less than 4^R probes (as set forth in claim 115), or wherein the double-stranded portion is about or is 3-20 nucleotides and the single-stranded portion is about or is 3-20 nucleotides (claims 72 and 73).

2. **The solid supports comprising the arrays as set out in claims 89-94 and 116 are not taught or suggested by the cited references.**

Claims

Claims 89-94 and 116 are directed to a solid support containing an array of nucleic acid probes that have a double-stranded portion and a single-stranded portion containing a random nucleotide sequence. Claims 89-94 specify that the random nucleotide sequence of the probes is of length R. Claim 116 specifies that the double-stranded portion of the probes comprises a constant sequence.

Differences between the claimed solid support and the cited references

The methods and compositions described in Khrapko and Drmanac are se out above in the discussion of the rejection with respect to claims directed to arrays of nucleic acid probes. As discussed above, because neither Khrapko nor Drmanac, alone or in combination, teach or suggest an array of probes containing a double-stranded portion and a single-stranded portion including a random sequence of length R or a double-stranded portion containing a constant sequence as claimed in the instant application the cited references cannot and do not teach or suggest a solid support comprising such an array of nucleic acid probes. Such a composition containing an array of nucleic acid molecules of the specific structure set forth in the claimed arrays simply is not taught, suggested or the result of the methods described in the cited references. Accordingly, because the combination of cited references fails to result in the claimed solid supports, they cannot be relied on to establish *prima facie* obviousness of the claimed compositions.

3. **The methods of creating an array of probes as set out in claims 1-5, 65-69, and 111-113 are not taught or suggested by the cited references.**

Claims

Claims 1-5, 65-69 and 111-113 are directed to methods of creating an array of probes. These claims specify that two sets of nucleic acids are synthesized: a first set of nucleic acids containing a constant sequence and a

random sequence, and a second set of nucleic acids each containing a sequence complementary to the constant sequence of the first nucleic acid. The first set is then hybridized with the second set to generate an array of probes having a double-stranded region and a single-stranded region containing the random sequence. Claims 1, 2, 5, and 65-69 specify that the double-stranded region comprises the constant sequence. Claims 111 and 112 specify that the random sequence is of length R. Claims 113, 3 and 4 specify that the constant sequence is of length C at a 3' terminus and that the random sequence is of length R at a 5' terminus.

Differences between the claimed methods of creating an array of probes and the cited references

As discussed above, Khrapko describes a technique, referred to as sequencing by hybridization with an oligonucleotide matrix (SHOM) containing single-stranded oligonucleotides and experiments to (1) test the method using a 17-nucleotide DNA fragment and (2) determine if continuous stacking hybridization (CSH) may increase the efficiency of SHOM. These experiments were not conducted to generate an array of probes having a double-stranded region and a single-stranded region containing a random sequence and nor do the methods yield such an array of probes. None of the hybridized matrices resulting from experimental procedures described in Khrapko contains an array of probes that is generated from the instantly claimed methods.

The procedure described in Khrapko for the experiment designed to test CSH, a 5-mer, a 17-mer and an immobilized 8-mer or 7-mer were simultaneously hybridized. Only one 5-mer sequence was used and only one 17-mer sequence was used. Three 8-mer sequences were used, which contain an identical core sequence but have different nucleotides on the 5' and/or 3' ends of the core sequence. Because each 5-mer was identical and each 17-mer was identical, neither of these sequences could be viewed as a "set of nucleic acids each comprising a constant sequence and a random sequence" because the

sequences of each were identical. The only nucleic acids of the three separate oligonucleotides involved in the simultaneous hybridization that were not identical were the 8-mers. Even if, for the sake of argument, the non-identical nucleotides of the 8-mers were considered "random" sequence, the so-called "random" sequence would not be contained in a single-stranded region of the resulting hybridization product, but rather in a double-stranded region. No matter how the several nucleic acids that were hybridized in this hybridization process described in Khrapko are characterized, the result of hybridization of the nucleic acids is **not** an array of probes having a double-stranded region and a single-stranded region containing a random sequence of length R or having a double-stranded region containing the constant sequence and a single-stranded region containing the random sequence. Therefore, the hybridization procedure described for CSH in Khrapko cannot be the same as the method for creating an array of probes claimed in claims 1-5, 65-69 and 111-113.

Drmanac describes immobilization of single-stranded probes to membranes or beads. The reference does not teach or suggest synthesizing two sets of nucleic acids each containing a complementary constant sequence that when hybridized to each other form an array of probes having a double-stranded region and a single-stranded region containing a random sequence of length R or a double-stranded region containing the constant sequence and a single-stranded region containing the random sequence.

The Office Action has failed to set forth a case of *prima facie* obviousness

Relevant law

Relevant law is set forth above. It is noted that all claim limitations must be taught or suggested by the prior art to establish that claims are *prima facie* obvious. *See, e.g.,* MPEP 2143.03 and *In re Gulack*, 703 F.2d 1381, 217 U.S.P.Q. 401 (Fed. Cir. 1983).

Analysis

As described above, neither Khrapko nor Drmanac teach or suggest a method of creating an array of probes that have (1) a double-stranded portion and a single-stranded portion containing a random nucleotide sequence of length R or (2) a double-stranded region containing a constant sequence and a single-stranded region containing a random sequence. Although the Office Action states with respect to the rejection of all the claims (except claim 3) that "CSH taught by Khrapko would not be possible without a partially single-stranded probe which is formed at the same time that the hybridization is carried out," it fails to provide any discussion of Drmanac, much less a discussion of how Drmanac might cure the above-noted defects of Khrapko as a reference against the claimed methods for creating an array of probes.

It appears that the Office Action relies solely on the actual CSH method described in Khrapko for the rejection of the claimed methods for creating an array of probes since it also fails to point out how either reference (Khrapko or Drmanac) may provide any motivation or suggestion to modify the hybridization process for CSH as described in Khrapko to yield the claimed method for creating an array of probes. If this is in fact the case, then it would seem that the claims are actually being rejected as anticipated by Khrapko under 35 U.S.C. §102(b). However, as described above, Khrapko fails to teach each element of the claimed methods and in fact describes a process that does not yield the product of the claimed method. Accordingly, Khrapko does not anticipate the claimed methods for creating an array of probes or any of the claimed compositions or other claimed methods.

Notwithstanding that the Office Action fails to establish a *prima facie* case of obviousness of the claimed methods, there must be some teaching or suggestion in the cited references or combination of the cited references to do what Applicant has done (*In re Fritch* 23 U.S.P.Q. 1780 (Fed. Cir. 1992)). In this instance there is no suggestion to hybridize two sets of nucleic acids, one

set of nucleic acids containing a constant sequence and a random sequence, and a second set of nucleic acids each containing a sequence complementary to the constant sequence of the first nucleic acid, to generate an array of probes having a double-stranded region and a single-stranded region containing the random sequence. Although hybridization reactions are described in the references, the methods, alone or in combination, do not yield an array of probes as set forth in the claimed methods for creating an array of probes and thus cannot be the same method as claimed in the instant claims. There is no suggestion or even hint to do what Applicant has done. Accordingly, the Office Action fails to establish a *prima facie* case of obviousness of the methods of claims 1 and 111 for creating an array of probes.

Furthermore, the cited references, alone or in combination, fail to yield the methods for creating an array of probes wherein the nucleic acids of the first of the two sets of nucleic acids to be hybridized are each between about 15-30 nucleotides and the nucleic acids of the second set are between about 10-25 nucleotides (claim 2), or wherein the resulting array is fixed to a solid support (claims 5, 65 and 66), or wherein the probes are labeled (claims 67 and 68) or wherein the nucleic acids are DNA, RNA or PNA (claim 69). In addition, neither of the cited references, alone or in combination teach or suggest methods of creating arrays of probes that contain a double-stranded region and a single-stranded region containing a random sequence of length R, as specified in claims 111 and 113, or wherein the array contains about 4^R probes (claims 4 and 112) or wherein R is between about 3-5 nucleotides and C, the length of a constant sequence in the nucleic acids of the first set of nucleic acids, is between about 7-20 nucleotides (claim 3). None of the hybridized matrices generated by the processes described in Khrapko or Drmanac contain hybrids which contain random sequences of equal length (i.e., length R) in a single-stranded region.

4. **The methods of sequencing a target nucleic acid as set out in claims 95-110 and 117-120 are not taught or suggested by the cited references.**

Claims

Claims 95-110 and 117-120 are directed to methods of sequencing a target nucleic acid and include steps of hybridizing target nucleic acid that is at least partly single-stranded to an array of nucleic acid probes that have a double-stranded portion and a single-stranded portion containing a random nucleotide sequence.

Differences between the claimed methods of sequencing and the cited references

Khrapko describes a technique for sequencing nucleic acids (referred to as SHOM) by hybridizing a target single-stranded nucleic acid sequence to **single-stranded** probes. This is made clear on page 375 (first full paragraph in the right column) where it is stated "[t]o illustrate Sequencing by Hybridization to Oligonucleotide Matrix (SHOM), let us take the simple example of a labeled fragment CTCA (TGAG as a complementary strand) and a matrix of the whole set of $4^3 = 64$ trinucleotides. The fragment will specifically hybridize only with complementary trinucleotides TGA and GAG..."

In experiments described in the reference to demonstrate the efficiency and fidelity of SHOM, a model 17-mer is used. In one experiment designed to generate and discriminate thermal dissociation curves for perfect and mismatched duplexes, single-stranded 8-mers were immobilized to a polyacrylamide-covered glass plate. Four single-stranded 17-mers differing by a single base substitution were separately hybridized to the immobilized 8-mers, each of which was complementary to a portion of one of the 17-mers. The hybridizations would thus form perfect as well as imperfect (single mismatches) duplexes.

It is suggested in Khrapko that continuous stacking hybridization (CSH) could increase the fidelity of SHOM by resolving ambiguities at branching points. As discussed above, the CSH method described in Khrapko is a

simultaneous hybridization of single-stranded target DNA with immobilized single-stranded octanucleotides and labeled selected single-stranded pentanucleotides to form a continuously stacked perfect duplex of 13 base pairs. In the nucleic acid sequencing method described in Khrapko, CSH is conducted as "additional hybridizations" after first hybridizing a single-stranded labeled target fragment with immobilized single-stranded octamers. In the CSH additional hybridizations, an unlabeled single-stranded target DNA fragment is hybridized with the single-stranded octamers **in the presence of** a **set** of labeled single-stranded pentanucleotides.

Khrapko does **not** teach or suggest a method of sequencing a nucleic acid by hybridization of an at least partly single-stranded target nucleic acid to an array of pre-existing probes having a double-stranded portion and a single-stranded portion containing a random nucleotide sequence. Target nucleic acid sequence in all sequencing methods described in Khrapko is always hybridized to a nucleic acid that is only single-stranded, be it a single-stranded immobilized octamer and/or a single-stranded pentanucleotide. Thus, contrary to the assertion in the Office Action that a "partially single-stranded **probe** is formed at the same time as hybridization is carried out" in CSH described in Khrapko, any "probe," i.e., the non-target nucleic acid to which a target nucleic acid to be sequenced hybridizes, is always single-stranded. The probe in the sequencing method of Khrapko is the immobilized 8-mer. The pentanucleotide used in CSH, which is not considered a "probe." However, even if the pentanucleotide is considered a probe to which a target nucleic acid sequence is hybridized, the pentanucleotide is also single-stranded. The only duplexed nucleic acids in the sequencing methods described in Khrapko are formed between a target nucleic acid hybridizing either to a single-stranded pentanucleotide or a single-stranded 8-mer probe. The target nucleic acid does not hybridize to a pre-existing probe that is partially single-stranded, partially double-stranded (double-stranded in the sense that the double-stranded molecule does **not** include a strand of the target

nucleic acid but is a double-stranded portion of the probe) in the sequencing methods of Khrapko.

Drmanac does not cure this deficiency. Drmanac, which does not describe a particular method of nucleic acid sequencing, provides a description of experiments designed to investigate possible DNA hybridization conditions that may permit discrimination between perfectly matched duplexes and duplexes with a single mismatch. Similar to Khrapko, hybridization methods described in Drmanac are methods in which a labeled short single-stranded oligonucleotide probe is hybridized to a single-stranded target nucleic acid (see, e.g., p. 528, left column, third full paragraph). Drmanac does not teach or suggest the use of probes that contain a single-stranded and double-stranded portion to hybridize to a target nucleic acid that is at least partially double-stranded. Drmanac does not suggest or provide any motivation for modifying the sequencing method described in Khrapko to include any type of molecule that has a double-stranded portion and a single-stranded portion containing a random sequence for hybridization to a target nucleic acid. Further, the reference does not expressly teach any method for sequencing a target nucleic acid. Instead this art teaches conditions necessary for optimal hybridization of **single-stranded** oligonucleotides to cloned DNA.

The Office Action has failed to set forth a case of *prima facie* obviousness

Relevant law

Relevant law is set forth above. It is noted that all claim limitations must be taught or suggested by the prior art to establish that claims are *prima facie* obvious. *See, e.g.,* MPEP 2143.03 and *In re Gulack*, 703 F.2d 1381, 217 U.S.P.Q. 401 (Fed. Cir. 1983).

Analysis

As described above, neither Khrapko nor Drmanac teach or suggest a method of sequencing a target nucleic acid by hybridizing a target nucleic acid that is at least partly single stranded to an array of nucleic acid probes having a

double-stranded portion and a single-stranded portion containing a random sequence. Although Khrapko describes a method of sequencing a target nucleic acid (SHOM and SHOM plus CSH), the single-stranded target nucleic is hybridized only to single-stranded molecules (i.e., an 8-mer probe and a pentanucleotide). There is simply not a step in the sequencing method of Khrapko in which a target nucleic acid is hybridized to anything other than a single-stranded nucleic acid molecule.

It appears that the Office Action relies solely on the actual CSH method described in Khrapko for the rejection of the claimed methods for sequencing a target nucleic acid. The Office Action fails to point out how either the Khrapko or Drmanac reference may provide any motivation or suggestion to modify the sequencing method as described in Khrapko to yield the claimed sequencing method. If this is in fact the case, then it would seem that the claims are actually being rejected as anticipated by Khrapko under 35 U.S.C. §102(b). However, as described above, Khrapko fails to teach each element of the claimed methods. Accordingly, Khrapko does not anticipate the claimed methods for sequencing a target nucleic acid.

In addition, the Office Action fails to establish a *prima facie* case of obviousness of the claimed sequencing methods. The combination of Khrapko and Drmanac does not result in the claimed methods of sequencing a target nucleic acid. Accordingly, the Office Action fails to establish a *prima facie* case of obviousness of the methods of claims 95 and 110 for sequencing a target nucleic acid.

Furthermore, the cited references, alone or in combination, fail to yield the sequencing methods wherein the target is hybridized to the probe (claim 96), or wherein a strand of the probe is enzymatically extended (claims 97-102), or wherein the array is fixed to a solid support (claims 104-106), or wherein the target nucleic acid or probes are labeled (claims 107 and 108) or wherein the nucleic acids are DNA, RNA or PNA (claim 95), or wherein the hybridization

pattern is analyzed by a computer (claim 109), or wherein the random sequence in the single-stranded portion of the probe is of length R (claims 117 and 119), or wherein the double-stranded portion of the probe contains a constant sequence (claims 118 and 120).

Therefore, because the cited references alone or in combination do not result in the instantly claimed methods of sequencing a nucleic acid, the Office e Action fails to set forth a *prima facie* case of obviousness.

5. The methods of detecting a target nucleic acid as set out in claims 80-88 and 121-122 are not taught or suggested by the cited references.

Claims

Claims 80-88 and 121-122 are directed to methods of detecting a target nucleic acid in a sample including steps of contacting the sample with an array of probes that have a double-stranded portion and a single-stranded portion containing a random nucleotide sequence and identifying hybrids to detect the target nucleic acid. Claim 121 specifies that the random nucleotide sequence is of length R. Claim 122 specifies that the double-stranded portion comprises a constant sequence.

Differences between the claimed subject matter and the teachings of the cited reference

As discussed above, Khrapko describes a method for DNA **sequencing** by hybridization of a single-stranded target DNA to a matrix containing single-stranded oligonucleotide probes and discerning perfect from imperfect duplexes by generating thermal dissociation curves for all hybridizing oligonucleotides. A pentanucleotide may also be included in additional CSH reactions to accomplish simultaneous hybridization of the single-stranded probe and the single-stranded pentanucleotide to the target. No where in Khrapko is it taught or suggested to hybridize a target DNA to an array of probes having a double-stranded portion and a single-stranded portion containing a random nucleotide sequence for sequencing of the target nucleic acid or for any purpose.

In contrast claims 80-88 and 121-122, which are directed to methods of

detecting a target nucleotide or target nucleic acid molecule and not to methods of sequencing, include a step of contacting a sample of nucleic acid with an array of probes of specified structure, i.e., having a double-stranded portion and a single-stranded portion containing a random sequence, and identifying hybrids to detect target nucleic acid.

Similar to Khrapko, hybridization methods described in Drmanac are methods in which a labeled short single-stranded oligonucleotide probe is hybridized to a single-stranded target nucleic acid (see, e.g., p. 528, left column, third full paragraph). Drmanac does not teach or suggest the use of probes that contain a single-stranded and double-stranded portion to hybridize to a target nucleic acid. Drmanac does not suggest or provide any motivation for modifying the sequencing method described in Khrapko to include any type of molecule that has a double-stranded portion and a single-stranded portion containing a random sequence for hybridization to a target nucleic acid.

The Office Action has failed to set forth a case of *prima facie* obviousness

Relevant law

Relevant law is set forth above. It is noted that all claim limitations must be taught or suggested by the prior art to establish that claims are *prima facie* obvious. *See, e.g.,* MPEP 2143.03 and *In re Gulack*, 703 F.2d 1381, 217 U.S.P.Q. 401 (Fed. Cir. 1983).

Analysis

Neither Khrapko nor Drmanac, alone or in combination, result in the claimed methods for detecting a target nucleic acid. Furthermore, neither Khrapko nor Drmanac provide any motivation or teaching to modify the hybridization methods described therein to yield the claimed methods for detecting a target nucleic acid. Therefore, the Office Action fails to establish a *prima facie* case for obviousness of the methods of claim 80. Furthermore, the cited references, alone or in combination, fail to yield the detection methods wherein the sample is of an animal tissue, *e.g.*, human, environmental

substance or manufacturing product or by-product (claims 81 and 82), or wherein a detected target nucleic acid is purified (claim 83), or wherein the array is fixed to a solid support (claims 84-86), or wherein the target nucleic acid or probes are labeled (claims 87 and 88), or wherein the random sequence in the single-stranded portion of the probe is of length R (claim 121), or wherein the double-stranded portion of the probe contains a constant sequence (claim 122).

* * *

In view of the remarks herein, reconsideration and allowance of the application are respectfully requested.

Respectfully submitted,
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